

XXIII.—*On some Disputed Points in Teleostean Embryology.*

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At the time when Balfour wrote his 'Comparative Embryology' less was known concerning the development of the Teleostei than concerning that of any other class of the Vertebrata. But since that time explorations in this field have been very numerous, and the results obtained have not only brought our knowledge of Teleostean development up to the level of that of the embryology of Elasmobranchs, but have in many cases given quite a new meaning to processes previously observed in the development of other types. Interpretations and conceptions that appeared satisfactory when founded on a comparison of Elasmobranchs, Amphibia, and Sauropsida have been found to be inconsistent with the phenomena presented by Teleostean ova, and have therefore had to be either modified or abandoned. But the absence of anything like sound criticism in biology allows all the numerous memoirs and papers that have been published on the subject during recent years to claim equal authority, although there is little agreement or harmony among them. My purpose in this paper is to draw attention to the points which have been firmly established by satisfactory evidence and to distinguish the sound from the unsound among recent descriptions and arguments.

*The Structure of the Mature Ovum.*

The ovum at the moment it leaves the oviduct of the female consists of the ovicell enveloped by a capsule or membrane which is everywhere in contact with it. The ovicell consists of a small quantity of protoplasm and a larger quantity of nutritive material or deutoplasm. In the usual type of pelagic ovum the deutoplasm forms a continuous homogeneous mass which is transparent, and the protoplasm forms a complete thin envelope around it. In many ova, *e. g.* those of many species of the Gadidæ and Pleuronectidæ, there is no other element in the ovicell than the pellucid yolk and the peripheral pellicle of protoplasm; but in many other pelagic ova, *e. g.* those of the gurnard (*Trigla*) and mackerel (*Scomber scomber*), there is in addition a somewhat large globule of oil. In some ova there are numerous oil-globules. Professor W. C. McIntosh, in his review of my 'Treatise on the Sole' (11),

says that I do not now hold the view that oil-globules occur in the perivitelline space. It is true that in a paper published in 1885, when I described the movement of the oil-globule of the egg of *Trigla gurnardus*, I was led into the error in supposing that the oil-globule moved between the naked surface of the yolk and the vitelline membrane. At that time I gave no further study to the ovum of the gurnard nor had I studied any other ovum containing oil-globules; but before the paper by Professors M'Intosh and E. E. Prince was published I had, in my paper on the development of Teleosteans occurring in the neighbourhood of Plymouth (4), already explained that the oil-globule in the ovum of the mackerel and the gurnard is situated within the thin pellicle of protoplasm which encloses the yolk. My words were:— "Thus it is evident that the yolk is to be regarded as a liquid enclosed within a layer of protoplasm continuous with the blastoderm, and at the surface of this liquid, next the protoplasmic layer, moves the oil-globule."

Of course these remarks of mine were intended to apply to *Scomber* and *Trigla* only, not to the relations of the oil-globules in all ova. But Prof. Prince, the pupil and fellow-worker of Prof. M'Intosh, in his paper on what he was pleased to call "oleaginous spheres" in Teleostean ova (8), stated that in some eggs the oil-globules occur outside the yolk *in the perivitelline space*. His words are (*loc. cit.* p. 88):— "The oil-globule in truth occupies different situations in different species, occurring within the yolk-mass or outside it in the perivitelline space, or rather in a fossa or pocket indenting the surface of the yolk. Examples of the latter condition are afforded by the Gadoid ovum studied by Hæckel and by *Motella mustela*, *Lophius piscatorius*, *Molva vulgaris*, and other forms." In the recent large memoir of Professors M'Intosh and Prince (9) I find no reference to or contradiction of this statement, and yet there can be little doubt that it is as erroneous as my own earlier remark concerning the oil-globule in *Trigla*—the truth being that in the cases mentioned by Prince the oil-globules are enveloped by a protoplasmic pellicle continuous with the protoplasmic layer which envelops the yolk and are therefore immovable. In fact in the case of *Trigla* and *Scomber* in the course of development the oil-globule becomes enveloped by the protoplasm of the periblast, and the periblast is formed by the increase in thickness of the original protoplasmic envelope of the yolk.

In all non-pelagic ova, and in some pelagic, the yolk itself, apart from the presence of oil-globules, is heterogeneous and discontinuous. It usually in this case consists of a large

number of yolk-spherules of various sizes, strictly comparable with the yolk-spherules of an Elasmobranch or Sauropsidan. In all cases where such separate yolk-spherules occur they are separated by strands and bands of protoplasm which are ultimately continuous with the protoplasm of the blastodisc and the cortical protoplasm of the vitellus.

I pointed out in my paper in the 'Journal of the Marine Biological Association' (4) that the continuous homogeneous yolk of the typical pelagic ovum was to be regarded as a single large yolk-sphere representing the numerous yolk-spherules of such an egg as that of the herring or salmon, fused together, all the protoplasm being thus driven to the external surface of the vitellus. And I also pointed out that eggs like that of the sole with a single external layer of yolk-segments formed an intermediate condition, in which, while most of the yolk-spherules had fused together, a few still remained separated by protoplasmic partitions. Development shows this to be the true account of the matter, for in the ovarian development of typical pelagic ova the homogeneous yolk is actually produced by the coalescence of distinct yolk-spherules. Agassiz and Whitman, in one of their beautiful memoirs (7), describe a layer of yolk-segments exactly similar to that in the sole, in an egg which they identify as that of *Temnodon saltator*. They fail to find any explanation of these yolk-segments, but they distinctly retract their previous conclusion that the segments indicated a partial segmentation of the yolk connected with the segmentation of the blastoderm, and partially representing the segmentation of the yolk in the Amphibian ovum. Examination of sections has shown them that these yolk-segments have nothing to do with either the segmentation of the blastoderm or the nuclei of the periblast.

In spite of this retraction by Agassiz and Whitman of their previous conclusion M'Intosh and Prince in their large memoir (9, p. 720) still refer to the yolk-segments in the eggs of *Temnodon saltator* and of the common sole as a rudimentary survival of the nucleated yolk-cells formed by the process of segmentation in the ova of Amphibians, *Petromyzon*, &c. The memoir of Agassiz and Whitman in which they repudiate this interpretation is mentioned by M'Intosh and Prince in their bibliographical list; but apparently Professor Prince, who we are told is responsible for the embryological part of the memoir, had not read all the works he refers to. Professor Prince does not allude to the obvious obstacle to any comparison between the yolk-segments in the egg of the sole and the yolk-cells of the Amphibian ovum, namely that the former are developed in the ovary long before fertili-

zation has occurred or segmentation has commenced. He even goes so far as to include the yolk-segments in pelagic Clupeoid ova, *e. g.* that of the sprat, in his interpretation. If he followed out this interpretation to its logical consequences he would have to maintain that segmentation in the Clupeoids and the sole proceeds to a great extent in the ovary before the egg is extruded and before it has been fertilized.

The capsule which contains the ovicell may be a single continuous layer or may consist of two more or less distinct layers. It has been usually held, in accordance with the definitions adopted by Balfour in his 'Comparative Embryology,' that a vitelline membrane is a primary egg-membrane formed in the ovary by the protoplasm of the ovum, while an egg-membrane formed by the follicular epithelium is to be called a chorion. The term *zona radiata* is, according to Balfour, to be applied to vitelline membranes which contain numerous radial pores. Thus it is clear that a vitelline membrane may or may not be a *zona radiata*, but a *zona radiata* is always a vitelline membrane. Therefore it was scarcely necessary for Prof. M'Intosh to say that in my 'Treatise on the Sole' I call the *zona radiata* the vitelline membrane.

But it must be remembered that it is customary to call the envelope of the Teleostean egg the vitelline membrane, although it has not yet been proved beyond a doubt that it is formed by the action of the protoplasm of the ovum. I have shown conclusively that the processes of the egg-capsule in *Myxine* are formed last in the development of that capsule in the ovary; the internal part of the capsule is formed first, and it increases in thickness by addition to the outer side. Now the action of the ovum itself could only add new material to the internal surface of the egg-capsule, not to the external surface. Therefore the egg-capsule in *Myxine* must be formed in part at least by the follicular epithelium. Since many Teleostean ova possess processes of the capsule similar to those of *Myxine*, *e. g.* *Belone*, the outer part of the envelope of these ova must also be formed by the follicular epithelium; for the processes are the last part of the egg-membrane to be developed, not the first. Whether in some ova the whole of the egg-membrane is developed by the egg from without inwards, or whether part is always derived from the egg and part from the follicular epithelium has not yet been proved. Meantime, where, as in the sole's ovum, a single membrane only is discernible, I see no objection to following the example of such writers as Hæckel, Kölliker, and Waldeyer, and calling it simply the vitelline membrane. The term *zona radiata* is

not a satisfactory one, for zona means a girdle or belt and not a hollow sphere like the membrane to which it is applied, while radiata certainly does not mean "provided with minute tubes vertical to the surfaces." The large memoir of Professors McIntosh and Prince (9) gives no fresh evidence as to the development of the vitelline membrane; the paper of Iwakawa (12) to which they refer is not conclusive, nor are Scharff's observations in his paper "On the Intra-Ovarian Egg of some Osseous Fishes" (13).

### Segmentation.

It will be convenient to take Balfour's summary of the then state of knowledge in his 'Comparative Embryology' (1885) as our starting-point in considering this question. At that time the process of segmentation had not been followed step by step from its beginning to its end. Balfour says:—"In hardened specimens a small cavity amongst the segmentation-spheres may be present at any early stage; but it is probably an artificial product, and in any case has nothing to do with the true segmentation-cavity, which does not appear till near the close of segmentation. The peripheral layer of granular matter continuous with the germinal disk does not undergo division, but it becomes during the segmentation specially thickened; and, while remaining thicker in this region, gradually grows inwards, so as to form a continuous subblastodermic layer. In this layer nuclei appear which are equivalent to those in the Elasmobranch ovum. A considerable number of these nuclei often become visible simultaneously, and they are usually believed to arise spontaneously, though this is still doubtful. Around these nuclei portions of protoplasm are segmented off, and cells are thus formed which enter the blastoderm and have nearly the same destination as the homologous cells of the Elasmobranch ovum. During the later stages of segmentation one end of the blastoderm becomes thickened and forms the embryonic swelling, and a cavity appears between the blastoderm and the yolk which is excentrically situated near the non-embryonic part of the blastoderm. This cavity is the true segmentation-cavity.

"In *Leuciscus rutilus* Bambeke describes a cavity as appearing in the middle of the blastoderm during the later stages of segmentation. From his figures it might be supposed that this cavity was equivalent to the segmentation-cavity of Elasmobranchs in its earliest condition; but Bambeke states that it disappears and has no connexion with

the true segmentation-cavity. Bambeke and other investigators have failed to recognize the homology of the segmentation-cavity in Teleostei, Elasmobranchii, Amphibia, &c."

In his account of segmentation in the general portion of his work Balfour says that simple segmentation leads to the formation of a hollow vesicle or blastosphere enclosing a central cavity, which is called the segmentation-cavity or cavity of von Baer. In German works this cavity is called sometimes "Furchungshöhle," sometimes "Keimhöhle;" and some English embryologists speak of it as the subgerminal cavity or germinal cavity.

In my paper "On the Relations of the Yolk to the Gastrula in Teleosteans" (2) I described the history and relations of the segmentation-cavity as they are seen in the living pelagic and transparent ova of the cod, haddock, and whiting. I showed there that the segmentation-cavity does not become visible as a distinct space between the centre of the blastoderm and the protoplasmic envelope of the yolk (periblast) until the commencement of the invagination or the appearance of the hypoblastic ring. I showed that the cavity never exists beneath the germinal ring nor beneath the embryonic or dorsal rudiment. I showed that in the process of the envelopment of the yolk by the blastoderm the whole of the germinal ring becomes used up in the formation and increase of the dorsal rudiment, and that the central part of the blastoderm with the segmentation-cavity beneath it comes to form the ventral portion or yolk-sac of the embryo and larva.

Before this paper of mine was written Agassiz and Whitman, in a paper "On the Development of some Pelagic Fish-Eggs" (6) had demonstrated very clearly and conclusively the exact nature of the processes which take place during segmentation in the pelagic ovum. Hoffmann (14) had previously asserted, and supported the assertion with beautiful ideal figures having no relation to reality, that the first nuclear division took place horizontally and produced two new nuclei, one vertically above the other, the upper giving rise afterwards to the cells of the blastoderm, the multiplication of the lower forming the nuclei of the unsegmented periblast. Agassiz and Whitman showed that up to the four-cell stage there is no distinction between periblast and blastoderm, the cells being continuous with one another below and externally with the protoplasmic pellicle which envelops the yolk. But at the sixteen-cell stage they showed that the four central cells have separated from a thin layer of protoplasm below which covers the yolk, and are thus definitely limited and defined on all sides, while the twelve marginal cells remain continuous with

both the subblastodermic protoplasmic layer and the protoplasmic envelope of the yolk outside the blastoderm. Thus the segmentation-cavity is *potentially* established at this early stage, though there is no actual space between the central cells of the blastoderm and the subblastodermic protoplasm. Both the four central cells and the twelve marginal cells now go on dividing, the former principally dividing in the horizontal plane so as to form several layers of cells. When a marginal nucleus divides it divides vertically, its two halves lying horizontally side by side; the internal half becomes completely separate and joins the blastoderm, while the external half remains continuous with the cortical protoplasm of the ovum. At a later stage the nuclei divide faster than the protoplasm, and while some cells are separated off from the marginal cell to join the blastoderm, the multiplying nuclei extend into the cortical protoplasm both internally beneath the blastoderm and externally outside the blastoderm. Thus the nucleated syncytium called the periblast comes to be established. Finally the centre of the blastoderm becomes thinner and lifts itself up from the subblastodermic periblast, and thus the actual segmentation-cavity is established. At the same time the cells which are constantly being separated off from the marginal periblast pass inwards and form a definite layer beneath the germinal ring.

Now let us turn to the account of these processes given by M'Intosh and Prince in their bulky memoir in the *Edinburgh Transactions* (9). They say that the blastodisc is formed by the segregation at one pole of protoplasm, which, moreover, constitutes a superficial and tenacious layer around the vitellus; and that this layer is itself derived by centrifugal transference from the scattered protoplasm mingled with the general matrix of the yolk. These authors entirely ignore the distinction which exists with regard to the relations of the protoplasm between pelagic eggs with a continuous yolk and other eggs with a discontinuous yolk made up of yolk-spheres. In the former case, as I have shown in my memoir "*On the Eggs and Larvæ of Teleosteans*" (5), all the protoplasm of the ovum at the time of deposition is cortical, and there is no "scattered protoplasm mingled with the general matrix of the yolk." In the herring's egg and all eggs with discontinuous yolk the yolk-spherules are contained in a network of protoplasm. In the former case the protoplasm collects to form the blastodisc only from the cortex; in the latter case it collects from the internal network as well. M'Intosh and Prince cite various authors in support of the idea that the protoplasm during development is nourished and grows at the expense of

he yolk ; but surely that fact is sufficiently obvious—what else is the yolk for ?

M'Intosh and Prince have not fully grasped the meaning of my remarks concerning the segmentation in my paper "On the Relations of the Yolk to the Gastrula." I said that the first cleavage of the blastodisc into two cells represented theoretically the division of the whole ovum into two similar cells, each containing a cap of protoplasm and a large quantity of yolk, although actually the two cells are continuous inferiorly and the yolk is continuous throughout. They say it is difficult to maintain such a relation of blastomeres and yolk when the morula is reached. By morula they apparently mean the segmented blastoderm. But it is obvious enough from my papers and from those of the most reliable embryologists that at this stage the same relation is maintained between the undivided nucleated periblast and the yolk which it envelops. These portions of the ovum together represent and are homologous with, as I said in the paper referred to, the nucleated yolk-cells of the frog's ovum.

M'Intosh and Prince deny the correctness of Agassiz and Whitman's statement that there is a definite separation during the later stages of segmentation between the central part of the blastoderm and the subblastodermic periblast. They say that the line of demarcation is broken in sections by knob-like processes which project from the blastoderm into the yolk ; but the figure they refer to in support of this statement does not include the yolk at all ! In fact the figures they give of sections of the blastoderm are quite unsatisfactory, and seem to indicate that the sections themselves were too imperfect to prove anything. At any rate my own sections of the ova of the mackerel at different stages of segmentation entirely confirm the results of Agassiz and Whitman so far as concerns the complete demarcation between the central cells of the blastoderm and the subblastodermic periblast. I differ, however, from those authors in denying that there is actually any space beneath the blastoderm in the living ovum ; the surface of the subblastodermic layer, though distinct from, is in contact with, the lower surface of the blastodermic cells.

I fully agree with M'Intosh and Prince when they state that a cavity appears between the under surface of the central portion of the blastoderm and the periblast after the stage of simple segmentation is completed. But they say, "We speak of it as a germinal cavity, and do so advisedly, for it is not the cavity of Von Baer, better known as the blastocœl or segmentation-cavity." It will probably be difficult to convince these authors that this cavity is, as Balfour and the



most reputed embryologists have always maintained, the same thing as the segmentation-cavity in other ova; for they do not seem to admit the fact that all ova are homologous, and that the various modes of development, leaving aside those exhibited by Cœlenterata and Crustacea, are modifications of one fundamental plan. But I would point out that the term germinal cavity is synonymous with segmentation-cavity, and that if they wish to maintain that the cavity in Teleostean ova is something else, they ought to give it some other name. These authors admit that Balfour's segmentation-cavity in Elasmobranchs is homologous with the segmentation-cavity of Amphibians, although, as they also admit and as Balfour states, the floor of the cavity in Elasmobranchs is at one stage formed by the yolk with its external protoplasmic layer, as in Teleostei. The basis of the surprising conclusion of M<sup>r</sup>Intosh and Prince is obvious enough. They speak of the Teleostean germ after segmentation as a morula which flattens out and becomes lifted up and separated by a chamber from the appended trophic mass. That is to say, they regard the yolk with its envelope of nucleated protoplasm as something distinct from the germ, and the germ, or, to use the proper term, the blastoderm, as alone homologous with the morula of an egg with simple equal segmentation, such as that of *Amphioxus*. But, as is satisfactorily shown by Balfour's *Comp. Embryology*, chap. xi. vol. ii., and by my paper "On the Relations of the Yolk to the Gastrula" (2), not to mention numerous other papers by different embryologists, the Teleostean egg must be compared whole for whole with any other egg. The yolk is not something added on to the outside of the egg, but is an accumulation of food-material within the egg itself. As development proceeds certain cells are separated, while others, namely the nucleated periblast, contain the whole yolk; and it is as certain as any other ascertained relation in embryology that the periblast and the yolk are homologous with the yolk-cells in the Amphibian ovum, which there form the floor of the segmentation-cavity. That this is so is conclusively proved by the fact, demonstrated in my paper on Kupffer's vesicle, that the periblast gives rise to cells which form the floor of the intestine, as do the yolk-cells in Amphibians. My account of Kupffer's vesicle, excepting that part which refers to the formation of the floor of the gut from the periblast, has been entirely confirmed by a paper published last year (15) by Henry V. Wilson in America.

In the light of the above considerations it is somewhat extraordinary that Prof. M<sup>r</sup>Intosh, in his review of my work on the sole (11), should write, "He prefers the term 'seg-

mental cavity' to Prof. Ed. E. Prince's less ambiguous term 'germinal cavity.'" The term I used was segmentation-cavity, not segmental cavity; and how anyone can maintain that that term, whose meaning has been firmly established by Balfour and every other leading embryologist, is more ambiguous than Prince's use of the term germinal cavity, utterly passes my comprehension.

But we cannot yet leave the account given by M'Intosh and Prince of the segmentation-cavity. They speak of another cavity observed in some Teleostean ova as representing the true blastocœl; but it is generally admitted by recent observers that there is but one cavity—that which M'Intosh and Prince call the germinal cavity, and it is this alone which I am discussing. Those authors proceed to argue that this cavity, having been, as they think, proved not to be the *blastocœl*, is really the *enterocœl* or cavity of invagination. It would take too much space to summarize their arguments. It will be sufficient to mention one or two facts which entirely disprove their conclusion. They say that the cavity is roofed over by endoderm- and epiblast-cells. It is a simple fact, which admits of no dispute, that the portion of the blastoderm which forms the roof of the cavity does not consist of endoderm at all, but wholly and exclusively of epiblast. The hypoblast or endoderm is represented by the lowest layer of the germinal ring and by certain cells derived at a later stage from the periblast; the germinal ring all goes to form the dorsal rudiment of the embryo. No part of the outer covering of M'Intosh and Prince's "germinal cavity" ever has anything whatever to do with the formation of the intestine, and therefore has nothing to do with the hypoblast. Now an enterocœl must be entirely surrounded by hypoblast; what, then, becomes of the extraordinary proposition of the St. Andrews embryologists?

#### *The Periblast.*

I have previously referred to the account given by Agassiz and Whitman (6) of the origin of the nucleated periblast. I fully accept their conclusions as to the origin of the first nuclei in that layer; but I consider that their figures indicate a different subsequent history of the layer from that which they describe in their text. I believe, as I have said before, that as the nuclei of the marginal cells from the sixteen-cell stage onwards continually divide, cell-division also takes place in these cells, but at a slower rate than the nuclear division. In consequence of this new cells are continually being sepa-

rated from the ring of periblast *at the same time that* the nuclei in that ring continually become more numerous and extend outwards and inwards from the marginal region of the blastoderm. Agassiz and Whitman do not admit that cells are separated from the periblastic layer after it is once formed. They admit apparently that the marginal cells of the blastoderm, which are continuous inferiorly with the sub-blastodermic and extra-blastodermic pellicle of protoplasm, continue to divide almost up to the appearance of the actual segmentation-cavity, and that the inner cells join the blastoderm while the outer remain continuous with the protoplasmic envelope of the yolk. But after this time, when the nucleated syncytial periblast is definitely formed, they say it remains at every stage so perfectly distinct from every other portion of the embryo that they see no ground for suspecting that it enters into any of the permanent embryonic layers. The hypoblast they believe to arise from the division and centripetal ingrowth of cells from the margin of the disk.

I believe myself, from a comparison of the various descriptions published and from my own observations, that some portion or other of the periblast is always, throughout its existence, budding off cells, which go to join the blastoderm or tissues of the embryo.

In this belief I agree to some extent with Brook. This observer, in his paper "On the Germinal Layers in Teleostei" (16), leaves the question of the first origin of the nuclei in the periblast open, his preparations of the herring's ovum not having afforded conclusive evidence on the subject. But he maintains that the hypoblast of the germinal ring is formed by cells segmented off from the nucleated periblast beneath the ring. I think he is most likely right, and therefore I have to abandon the view I formerly took, that the hypoblast layer was formed solely by a centripetal ingrowth from the edge of the blastoderm. Of course my general view of the morphological significance of the "invagination" in the Teleostean ovum remains unaltered.

Next we have my own conclusions concerning the segmenting off of cells from the periblast at a much later stage to form the floor of the intestine in the region of Kupffer's vesicle (1); my views on this point I see as yet no reason to change.

Again, in the late embryonic history of the mackerel the large oil-globule becomes entirely surrounded by periblast. No ingrowth of cells from any part of the embryo ever occurs between the oil-globule and the periblast, yet nevertheless stellate chromatophores exactly similar to those of the mesoblast of the skin make their appearance on the deep internal

surface of the oil-globule. These pigment-cells must have been derived from the periblast, and afford an undeniable example of mesoblastic cells formed directly from that layer. The occurrence and significance of these chromatophores was, I believe, first pointed out by John A. Ryder (17), in the 'American Naturalist,' Extra, November 1886; they have also been described and discussed by myself in my paper in the Journ. Mar. Biol. Assoc. (4). In the same paper I described the occurrence of black chromatophores at the surface of the periblast in the embryo of *Pleuronectes microcephalus*; these pigment-cells are also developed directly from the periblast, no extension of the lateral mesoblast over the yolk having taken place.

Finally, it has been maintained by Ryder and others that colourless blood-cells are segmented off from the periblast and enter the venous sinus and heart. I have not observed this myself; but there seems to be good evidence for its occurrence.

Thus the periblast with the yolk is evidently equivalent to the yolk-cells of the Amphibian ovum, and, like those cells, continues throughout development to take part in the segmentation, though in a different fashion, a part of the periblast remaining unsegmented, although nucleated, until the final absorption of the yolk. Both the yolk-cells and the periblast may be termed after a certain stage primitive hypoblast, provided it be remembered that a great part of the primitive hypoblast gives rise to mesoblastic cells. This last fact is well brought out by Shipley in his paper "On the Development of *Petromyzon*," where he says:—"The first formation of the mesoblastic plates appears to take place by a differentiation of the hypoblastic yolk-cells *in situ*, and not from invaginated cells." This differentiation consists chiefly in subdivision, by which small cells of the mesoblast are produced from the large yolk-cells. In fact a little consideration shows that the segmentation of cells from the Teleostean periblast to form hypoblast and mesoblastic tissues corresponds perfectly with the subdivision of the yolk-cells in *Petromyzon* and Amphibians which gives rise to hypoblast and mesoblast in those forms.

#### *Relation of Oil-globules to Periblast.*

As I have already said, in the mature undeveloped ovum oil-globules are sometimes free in the yolk, sometimes fixed by envelopment in the cortical protoplasm of the egg. But in all cases, so far as my experience goes, the oil-globules are during deve-

lopment enveloped by the protoplasm of the periblast. A very interesting movement of the oil-globules during development is seen in that of the sole's ovum. The small oil-globules here are enveloped by the cortical protoplasm, and nearly all of them are at first situated in an irregular ring of groups not far from the edge of the blastoderm. As the blastoderm advances the periblastic protoplasm is continually increasing in thickness and extent by assimilation of the yolk, and when the ring of oil-globules is overtaken by the advancing periblast beneath the blastoderm it is carried along bodily in the advance of the periblast, so that ultimately the groups of oil-globules are translated to a position beneath the lateral region of the embryonic dorsal rudiment. Nothing could illustrate more beautifully the fact that the embryonic dorsal rudiment is formed by the conrescence of the two halves of the germinal ring. This fact alone proves the truth of the theory of conrescence. Prof. M'Intosh, in his review (11), says that I now locate the oil-globules of the sole's ovum beneath the trunk of the embryo sole. But the position in which I have represented them in my 'Treatise' is the same as that in which I represented them in my paper in the 'Journal of the Marine Biological Association' in 1889. M'Intosh and Prince, in their memoir (9), say that the subsequent arrangement of the oil-globules under the developing embryo indicates probably that something like a streaming of the protoplasm of the periblast takes place about the period of the closure of the blastopore, so as to carry the globules under the developing embryo. It is rare that the globules ever lie beneath the axial region of the embryonic rudiment, and the supposed streaming of the protoplasm is merely the coalescence of the edges of the germinal ring, with its subjacent periblast, to form the embryonic dorsal rudiment. The vesicular layer of the yolk in the sole's egg extends *pari passu* with the extension of the periblast and blastoderm.

#### *Later History of the Segmentation-Cavity, Formation of the Heart, &c.*

As the blastoderm gradually increases in extent and grows over the yolk the segmentation-cavity also becomes much extended, and separates the epiblast from the periblast everywhere except beneath the embryonic shield and germinal ring. It must be remembered that sections show that the mesoblast layer is entirely confined to the embryonic shield and germinal ring, at least until the closure of the blastopore. In eggs such as those of the Salmonidæ and those of *Cyclopterus*, *Cottus*, &c.,

in fact in all eggs which exhibit vitelline vessels, the segmentation-cavity is obliterated after the closure of the blastopore by the production of mesoblast between the epiblast and periblast. This mesoblast is produced partly by the extension of the mesoblastic layer from the lateral region of the dorsal embryonic rudiment, partly, I believe, by the formation of mesoblastic cells from the periblast. In the mesoblast thus produced around the yolk, tubes are hollowed out to form the vitelline arteries and veins, the veins becoming continuous with the cavity of the heart, which is formed in a similar way in the mesoblast ventral to the pharynx.

In pelagic ova and in the ova of the herring the course of affairs is somewhat different. In these ova and the larvæ hatched from them there are no vitelline blood-vessels. In them the segmentation-cavity may, and probably does, disappear for a time after the closure of the blastopore in consequence of its epiblastic and periblastic walls coming into contact. But the lateral mesoblastic plates do not extend into it and obliterate it. When the heart commences to be formed the segmentation-cavity seems to reappear; that is to say, a cavity appears between the periblast and the epiblast of the yolk-sac. This cavity is continuous all round the ventral region and sides of the yolk, and anteriorly it is in communication by a definite large aperture with the posterior end of the auricle of the heart. But this cavity is theoretically no longer the segmentation-cavity; it is, at least on its inner or periblastic side, partially lined by mesoblastic cells, namely chromatophores produced from the periblast. Morphologically, as I have pointed out in my paper in the 'Journal of the Marine Biological Association' (4), this cavity is homologous with the vitelline blood-vessels in the salmon embryo, and, like those vessels, it is continuous with the auricle of the heart. It is shut off from the pericardium by a definite continuous mesoblastic membrane, and it is also completely separated from the body-cavity formed in the mesoblast at the sides of the embryo. It is not till a late stage of development, namely when the yolk has been entirely absorbed, that the mesoblast is sufficiently developed ventrally to divide up this perivitelline blood-sinus into separate blood-vessels, the blood-vessels which in the adult form the veins and arteries of the viscera.

I claim the credit of having been the first to give this explanation of the fact that in pelagic ova the heart is in open communication posteriorly with a continuous cavity round the yolk, a cavity which appears to be the segmentation-cavity. Shipley (19) has shown that an exactly similar

relation exists in *Petromyzon*; but he merely says that the perivitelline space is part of the segmentation-cavity, and that it subsequently becomes shut off by the downgrowth of the mesoblast, and forms the subintestinal vein. He makes no comparison between the perivitelline blood-sinus and the vitelline blood-vessels of other forms.

If M'Intosh and Prince had really understood the later history of the segmentation-cavity they would never have argued that it was the gastrula-cavity. The gastrula-cavity must by its definition become the lumen of the intestine, and the segmentation- or "germinal" cavity never has any connexion with the lumen of the intestine. I have shown that the real representative of the gastrula-cavity in Teleosteans is Kupffer's vesicle.

Ryder in 1884 (18) was of opinion that the segmentation-cavity in the later stages was "synonymous" with the body-cavity; whether he still holds this opinion I do not know, but it is evident from the above that it is entirely erroneous; the segmentation-cavity has as little to do with the body-cavity as it has with the gastrula-cavity. The apparent continuity in development of the segmentation-cavity with the perivitelline blood-sinus is due entirely to the retarded development of the mesoblast in pelagic ova and certain others; while the obliteration of the segmentation-cavity by the mesoblast, which takes place in Amphibian and many Teleostean ova, is represented in pelagic Teleostean ova by the formation of mesoblastic cells from the periblast. The cavity ceases to be a segmentation-cavity and becomes a perivitelline blood-sinus as soon as any definite mesoblastic cells are produced on its inner wall.

The account given by M'Intosh and Prince (9) of the development of the heart is, as a whole, to me quite incomprehensible, while many of the separate statements in that account are, I venture to say, erroneous. One of these statements is that "the heart usually pushes down before it a delicate stratum of hypoblastic cells; but this limiting ventral layer apparently becomes obliterated anteriorly, and the pericardial chamber is open to the subembryonic space, which is undoubtedly the persisting germinal cavity." This is the most extraordinary confusion. What has the hypoblast to do with the formation of the heart? Is it conceivable that the germinal cavity, which, according to these authors, is the gastrula or intestinal cavity, can be open to the pericardial cavity? Of course it is known that in certain forms the original gastrula-cavity segments off portions which form the body-cavity—*Amphioxus*, for instance. But in Teleosteans

the body-cavity is formed as a "schizocœl," and never has any connexion with either the lumen of the intestine or the gastrula-cavity or the segmentation-cavity. In fact I can state, from my own observations, that the pericardial cavity has no communication with the perivitelline cavity; and the same conclusion is confirmed by Shipley's observations on *Petromyzon*. In the nine quarto pages which M'Intosh and Prince devote to the development of the heart and blood-vessels I can find nowhere any mention of the fact that the auricle is open posteriorly to the perivitelline blood-sinus, which has the same position as the segmentation-cavity of an earlier stage. It seems as though these observers had either overlooked the posterior opening of the heart or had mistaken it for an opening into the pericardium\*.

#### *Chromatophores.*

Chromatophores may be developed both in larval and adult Teleosteans in other parts of the mesoblastic tissues besides the derma; but as a rule the coloration of the fish depends chiefly on the chromatophores present in the skin. In all the species which have come under my own observation the chromatophores in the skin of the larva or embryo at their earliest appearance are in all respects similar to those of the adult both in colour and in structure. For instance, in the adult mackerel there are black chromatophores and green chromatophores, and in the larva also black and green chromatophores appear. Similarly in the Pleuronectidæ the colour of the adult depends on the distribution of the black and yellow or orange chromatophores. I am leaving the iridocytes, whose colour-effect does not depend on pigment, out of consideration. In larval flat-fishes the only chromatophores developed are the black and the yellow or orange. Professor M'Intosh, in his review of my book on the sole, says, "The pigment of the larval sole in Scotland appears to differ materially from that of the larval sole at Plymouth, since it is not truly yellow, but dull stone-grey or dull yellowish white, and this afterwards changes into the ochreous hue so charac-

\* It must be added here that what I and most other writers on this subject have called the "pericardium" in the Teleostean embryo or larva is not exactly the same thing as the pericardium of the adult. I believe that the embryonic pericardium is merely a portion of the general coelom or body-cavity, the first part of that cavity to be developed ventrally. I believe that as the yolk disappears this embryonic pericardium extends backwards and becomes continuous with the lateral body-cavities, the adult pericardium being afterwards separated from the general body-cavity.



teristic of the post-larval sole." This seems to me a disingenuous style of criticism, unless, indeed, Prof. M'Intosh really believes that the larval sole has different colours in different places. The latter alternative is improbable; and if my reviewer thinks it more polite to suggest indirectly that I have blundered than candidly to express a doubt of the accuracy of my observation, I can only say that I do not agree with him. However, the discrepancy between our descriptions of the colour is easily explained. The yellow chromatophores of the larval sole do appear stone-grey, and often quite a silvery grey, when seen by reflected light either with the unaided eye or with the microscope; but when seen by transmitted light under the microscope they are yellow. The difference between these scattered larval chromatophores and those of the adult is merely due to the smaller quantity of the pigment and the absence of the opaque iridocytes from the skin in the larva. As to the post-larval sole referred to by Prof. M'Intosh and figured in the Scottish Fishery Board Report, 1889 (10), I cannot without further evidence accept the identification. It is not strictly speaking post-larval; all stages up to the commencement of the metamorphosis, that is all symmetrical stages, should be called larval. I have seen larvæ of the sole of a corresponding stage, as well as in subsequent stages, and in all the anterior margin of the head is much blunter and the mouth much more ventral than in Prof. M'Intosh's figure.

#### *Development of the Generative Organs.*

Hector F. E. Jungersen, in a masterly paper, has recently given a very complete and interesting account of the development of the ovaries and testes in Teleosteans. He has shown that in the Physoclist forms which he investigated the cavity of the ovary originates somewhat as McLeod described in *Belone*, namely by the formation of a groove on the surface of the genital ridge and the subsequent closing of this groove to form a canal. He has also shown that in the Physostomous forms, namely Cyprinoids, the cavity of the closed ovary is formed in the same way as that observed by me in *Clupea sprattus*, that is, not by the formation of a groove in the genital ridge, but by the coalescence of the lower edge of the genital ridge with another thinner ridge which projects from the peritoneum on the outer or lateral side of the genital ridge. Jungersen describes the genital cells as appearing in the mesoblastic tissue at the dorsal side of the body-cavity on either side of the mesentery. He shows

that this tissue is not properly speaking an epithelium, but a mass of cells of two kinds, namely genital cells distinguished by their large size and what he calls stroma-cells. Jungersen represents the genital cells at all stages, although they multiply by division, as uninucleate.

M'Intosh and Prince have not included the later development of the generative organs in their researches; but they give an account of the genital cells at the earliest stage which is in striking contrast to any previously given. They state that each of the primitive ova is "a more or less perfect sphere and encloses numerous minute nucleated bodies." They point out that these ova are quite unlike the primitive ova of Elasmobranchs as described by Balfour, for these latter are uninucleate. It is obvious that these authors have either misunderstood the structure of the primitive ova which they saw, or the multinucleate cells they describe were not primitive ova at all, but something else. It is at all events certain that the primitive ova of Teleosteans are, like the ova of most other animals, large cells with a single large nucleus.

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XXIV.—Notes on the Synonymy of some Species of Scolopendridæ, with Descriptions of new Genera and Species of the Group. By R. I. POCKOCK, of the British Museum (Natural History).

[Concluded from p. 68.]

[Plate V.]

ARTHRORHABDUS, gen. nov.

(ἄρθρον, a joint, and ῥάβδος, a staff.)

*Head-plate* entire, without sulci, not covered posteriorly by the first dorsal plate, but separated from it mesially by a space occupied by a horny membrane, which is united to the first tergite and appears to represent the basal plate.

*Eyes, mouth-parts, and stigmata* as in *Cormocephalus*.

*Anal somite* resembling that of *Cupipes*, the pleuræ being truncate, the legs stout, and the claw long and serrate.

Other characters, which may or may not prove to be of